93: Int J Cancer. 2003 Oct 10;106(6):848-55. Related Articles, Links

FULL TEXT AVAILABLE ONLINE from InterScience

Vascular endothelial growth factor expression correlates with matrix metalloproteinases MT1-MMP, MMP-2 and MMP-9 in human glioblastomas.

Munaut C, Noel A, Hougrand O, Foidart JM, Boniver J, Deprez M.

Laboratory of Tumour and Development Biology, University of Liege, Liege, Belgium.

Vascular endothelial growth factor (VEGF) is the major endothelial mitogen in central nervous system neoplasms and it is expressed in 64-95% of glioblastomas (GBMs). Tumour cells are the main source of VEGF in GBMs whereas VEGF receptors (VEGFR-1, its soluble form sVEGFR-1, VEGFR-2 and neuropilin-1) are expressed predominantly by endothelial cells. Infiltrating tumour cells and newly-formed capillaries progress through the extracellular matrix by local proteolysis involving matrix metalloproteinases (MMPs). Recent studies have shown that VEGF expression and bioavailability can be modulated by MMPs. We reported previously that the expression of MT1-MMP in human breast cancer cells was associated with an enhanced VEGF expression. We used quantitative RT-PCR, Western blot, gelatin zymography and immunohistochemistry to study the expression of VEGF, VEGFR-1, VEGFR-2, sVEGFR-1, neuropilin-1, MT1-MMP, MMP-2, MMP-9 and TIMP-2 in 20 human GBMs and 5 normal brains. The expression of these MMPs was markedly increased in most GBMs with excellent correlation between mRNA and protein levels; activated forms of MMP-2 and MMP-9 were present in 8/18 and 7/18 of GBMs. A majority of GBMs (17/20) also expressed high levels of VEGF, as previously reported, with strong correlation between VEGF and MT1-MMP gene expression levels, and double immunostaining showed that VEGF and MT1-MMP peptides co-localize in tumour and endothelial cells. Our results suggest that the interplay between metalloproteinases and VEGF previously described in experimental tumours may also be operative in human GBMs. Because of its dual ability to activate MMP-2 and to up-regulate VEGF, MT1-MMP might be of central importance in the growth of GBMs and represent an interesting target for anti-cancer treatments.

PMID: 12918061 [PubMed - indexed for MEDLINE]

Int. J. Cancer: 106, 848–855 (2003)

Publication of the International Union Against Cancer

VASCULAR ENDOTHELIAL GROWTH FACTOR EXPRESSION CORRELATES WITH MATRIX METALLOPROTEINASES MT1-MMP, MMP-2 AND MMP-9 IN HUMAN GLIOBLASTOMAS

Carine MUNAUT[1], Agnès NOËL[1], Olivier HOUGRAND[2], Jean-Michel FOIDART[1], Jacques BONIVER[2] and Manuel DEPREZ[2]*

[1]*Laboratory of Tumour and Development Biology, University of Liège, Liège, Belgium*
[2]*Laboratory of Neuropathology, University of Liège, Liège, Belgium*

Vascular endothelial growth factor (VEGF) is the major endothelial mitogen in central nervous system neoplasms and it is expressed in 64–95% of glioblastomas (GBMs). Tumour cells are the main source of VEGF in GBMs whereas VEGF receptors (VEGFR-1, its soluble form sVEGFR-1, VEGFR-2 and neuropilin-1) are expressed predominantly by endothelial cells. Infiltrating tumour cells and newly-formed capillaries progress through the extracellular matrix by local proteolysis involving matrix metalloproteinases (MMPs). Recent studies have shown that VEGF expression and bioavailability can be modulated by MMPs. We reported previously that the expression of MT1-MMP in human breast cancer cells was associated with an enhanced VEGF expression. We used quantitative RT-PCR, Western blot, gelatin zymography and immunohistochemistry to study the expression of VEGF, VEGFR-1, VEGFR-2, sVEGFR-1, neuropilin-1, MT1-MMP, MMP-2, MMP-9 and TIMP-2 in 20 human GBMs and 5 normal brains. The expression of these MMPs was markedly increased in most GBMs with excellent correlation between mRNA and protein levels; activated forms of MMP-2 and MMP-9 were present in 8/18 and 7/18 of GBMs. A majority of GBMs (17/20) also expressed high levels of VEGF, as previously reported, with strong correlation between VEGF and MT1-MMP gene expression levels, and double immunostaining showed that VEGF and MT1-MMP peptides co-localize in tumour and endothelial cells. Our results suggest that the interplay between metalloproteinases and VEGF previously described in experimental tumours may also be operative in human GBMs. Because of its dual ability to activate MMP-2 and to up-regulate VEGF, MT1-MMP might be of central importance in the growth of GBMs and represent an interesting target for anti-cancer treatments.

Key words: *VEGF; MMPs; glioblastomas; brain; tumor*

Angiogenesis is critical for the development of normal tissue and solid tumours. This process includes the degradation of the extracellular matrix (ECM) and the proliferation, migration and differentiation of endothelial cells, and is finely regulated by inhibitory and promoting factors.[1,2] Among positive factors, vascular endothelial growth factor (VEGF) has been proposed as the major endothelial mitogen in central nervous system (CNS) neoplasms.[3] Strong VEGF expression has been detected by immunohistochemistry in 64–95% of glioblastomas (GBMs).[4–6] GBMs are the most common malignant primitive tumours of the CNS in adults.[7] Microvascular proliferation is characteristic of these tumours and is an essential WHO diagnostic criteria.[7] Tumour cells are the main source of VEGF in GBMs whereas VEGF receptors are predominantly expressed by endothelial cells.[8,9] These receptors differ both in terms of affinity and transduction signaling.[10–12] VEGFR-1 (flt-1) and VEGFR-2 (flk/KDR) belong to the Class II tyrosine-kinase receptor family. VEGFR-1 has a soluble isoform (sVEGFR-1) that modulates VEGF availability.[8,13] Neuropilin-1 (NRP1) is a co-receptor for VEGF that increases by 10-fold the affinity of the $VEGF_{165}$ isoform for VEGFR-2.[14] NRP1 is thought to modulate VEGF-mediated tumour angiogenesis in human malignant astrocytomas.[10] The coordinated up-regulation of VEGF and its receptors appears as a critical event in the control of angiogenesis.[8,12]

Infiltrating tumour cells and newly-formed capillaries progress through the ECM by local proteolysis involving matrix metalloproteinases (MMPs).[15,16] MMPs are proteolytic enzymes that are synthesized as inactive zymogens. Their activation requires the removal of a propeptide by proteinase cleavage and can be inhibited by various tissue inhibitors of MMPs (TIMPs). Most MMPs are secreted as soluble enzymes but a subset of them are inserted in the cell membrane by a transmembrane domain or by a glycosylphosphatatidyl-inositol anchor and are classified as membrane-type MMPs (MT-MMPs).[17,18] In cultured tumour cells, MT-MMPs tend to accumulate on the cytoplasmic membrane of invadopodia where they selectively mediate local peri-cellular proteolysis. GBMs express high levels of MT1-MMP, MMP-2 and MMP-9.[19,20] Among these MMPs, MT1-MMP might play a central role in the remodeling of the ECM as this membrane-bound protease is able to activate MMP-2 and MMP-13.[21,22] Moreover, MT1-MMP has been shown to promote cell migration in various carcinoma cell lines by its ability to cleave laminin-5, a major constituent of basement membrane,[23,24] and through the processing of CD44H (the major receptor for hyaluronan)[25] and of $\alpha_v\beta_3$ integrin.[26]

MT1-MMP is involved in both developmental and tumour angiogenesis.[27] MT1-MMP overexpression in human melanoma cells has been associated with enhanced *in vitro* invasion and increased *in vivo* tumour growth and vascularization.[28] We have shown previously that in MCF-7 breast cancer cells, VEGF transcription is upregulated when MT1-MMP is overexpressed.[29] Up-regulation of VEGF by MT1-MMP has also been reported in a model of human glioma xenograft by Deryugina *et al.*[30] These experimental data suggest a link between MT1-MMP and the VEGF network. We have tested for the presence of such a link in human glioblastomas. We compared the expression of VEGF and its receptors (VEGFR-1, sVEGFR-1, VEGFR-2, NRP1) with

Grant sponsor: Communauté Française de Belgique (Actions de Recherches Concertées); Grant sponsor: Commission of European Communities; Grant sponsor: Fonds de la Recherche Scientifique Médicale; Grant sponsor: Fonds National de la Recherche Scientifique (FNRS, Belgium); Grant sponsor: Fédération Belge Contre le Cancer; Grant sponsor: Fonds Spéciaux de la Recherche (University of Liège); Grant sponsor: Centre Anticancéreux près l'Université de Liège; Grant sponsor: FB Assurances; Grant sponsor: Fondation Léon Frédéricq (University of Liège); Grant sponsor: D.G.T.R.E. from the "Région Wallonne"; Grant sponsor: Fonds d'Investissements de la Recherche Scientifique (CHU, Liège, Belgium); Grant sponsor: Federal Office for Scientific, Technical and Cultural Affairs (Brussels, Belgium).

*Correspondence to: Laboratory of Neuropathology, Tower of Pathology B23/1, B-4000 Sart Tilman (Liège), Belgium. Fax: +32-4-3662919. E-mail: Manuel.Deprez@ulg.ac.be

Received 23 October 2002; Revised 12 March 2003; Accepted 27 April 2003

DOI 10.1002/ijc.11313
Published online 26 June 2003 in Wiley InterScience (www.interscience.wiley.com).

MT1-MMP, MMP-2, MMP-9 and TIMP-2 in a series of 20 GBMs and 5 normal brains. Using quantitative RT-PCR, gelatin zymography, Western blot and immunohistochemistry, we showed a strong correlation between the expression of VEGF, MT1-MMP, MMP-2 and MMP-9 in GBMs. These results are in accordance with previous *in vitro* studies and add to the evidence of an interplay between VEGF and MMPs in the progression of human GBMs.

MATERIAL AND METHODS

Patients

We studied 20 GBMs diagnosed at the Laboratory of Neuropathology-CHU Liège between 1997 and 2001. The series included 17 primary GBMs (*i.e.*, no previous history of lower grade diffuse astrocytoma) and 3 secondary GBMs (*i.e.*, previous history of lower grade diffuse astrocytoma). Clinical information on these 20 cases have been reported previously as part of a larger series.[31] The gender ratio was 1/1, and the age at time of diagnosis ranged from 41–79 years (mean 56 years). Normal brain cortex and white matter were obtained from 5 patients with intractable epilepsy treated by partial temporal lobectomy. Histological examination of these specimens showed severe hippocampal sclerosis; frozen tissue was sampled from microscopically normal inferior temporal gyri. Our study was approved by the Ethical Committee of the Faculty of Medicine of the University of Liège.

RNA extraction and cDNA synthesis

Total RNA was extracted from cryosections with RNeasy Mini Kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's protocol. Total RNA (1 μg) was reverse transcribed with a ThermoScript reverse transcriptase (ThermoScript RT-PCR System, Invitrogen, Carlsbad, CA) and random hexamers as primers.

Primers

Primers pairs used in our study are described in Table I. Primers for the VEGF gene were chosen to distinguish between $VEGF_{189}$, $VEGF_{165}$, $VEGF_{145}$ and $VEGF_{121}$ mRNA isoforms. Intron-spanning primers and probes for the TaqMan system (primers for VEGFR-1 (Flt-1), sVEGFR-1, VEGFR-2 (KDR/Flk-1) and NRP1) were designed to meet specific criteria by using Primer Express software (Perkin Elmer, Foster City, CA). All primers were synthesized by Eurogentec (Liège, Belgium). The 5'- and 3'-end nucleotides of the probe were labeled with a reporter (FAM = 6-carboxy-fluorescein) and a quencher dye (TAMRA = 6-carboxy-tetramethylrhodamine). We conducted BLASTn (National Center for Biotechnology Information, Bethesda) searches against dbEST and the non redundant set of GenBank, EMBL, and DDBJ database sequences to confirm the total gene specificity of the nucleotide sequences chosen for the primers. The specificity of the amplified PCR products was confirmed either by restriction digest or by sequencing. The 18S ribosomal RNA was measured using the Pre-Developed TaqMan Assay Reagents Endogenous control kit from Applied Biosystems (Foster City, CA).

TABLE I – SEQUENCE OF PRIMERS AND TaqMan PROBES USED FOR RT-PCR STUDIES

Gene and accession number	Position	Sequence	Size	Cycles
MMP-2 FP	1740F	5'-AGATCTTCTTCTTCAAGGACCGGTT-3'	225 bp	33
MMP-2-RP	1964R	5'-GGCTGGTCAGTGGCTTGGGGTA-3'		
NM_004530				
MMP-9-FP	1592F	5'-GCGGAGATTGGGAACCAGCTGTA-3'	208 bp	37
MMP-9-RP	1800R	5'-GACGCGCCTGTGTACACCCACA-3'		
J05070				
MMP-14-FP	1288F	5'-GGATACCCAATGCCCATTGGCCA-3'	221 bp	32
MMP-14-RP	1508R	5'-CCATTGGGCATCCAGAAGAGAGC-3'		
NM_004995				
TIMP1-FP	78F	5'-CATCCTGTTGTTGCTGTGGCTGAT-3'	168 bp	33
TIMP1-RP	245R	5'-GTCATCTTGATCTCATAACGCTGG-3'		
M12670				
TIMP-2-FP	78F	5'-CTCGCTGGACGTTGGAGGAAAGAA-3'	155 bp	30
TIMP-2-RP	245R	5'-AGCCCATCTGGTACCTGTGGTTCA-3'		
NM 003255				
VEGF-FP	1208F	5'-CCTGGTGGACATCTTCCAGGAGTA-3'	479 bp	33
VEGF-RP	1687R	5'-CTCACCGCCTCGGCTTGTCACA-3'	407 bp	
AH001553			347 bp	
			275 bp	
28S rRNA-RP	12403F	5'-GTTCACCCACTAATAGGGAACGTGA-3'	212 bp	19
28S rRNA-RP	12614R	5'-GATTCTGACTTAGAGGCGTTCAGT-3'		
U13369				
VEGFR1-FP	2438F	5'-TCCCTTATGATGCCAGCAAGT-3'	79 bp	40
VEGFR1-RP	2516R	5'-CCAAAAGCCCCTCTTCCAA-3'		
VEGFR1 Probe	2469	5'-CCGGGAGAGACTTAAACTGGGCAAATCA-3'		
AF063657				
sVEGFR1-FP	2209F	5'-ACAATCAGAGGTGAGCACTGCAA-3'	180 bp	40
sVEGFR1-RP	2388R	5'-TCCGAGCCTGAAAGTTAGCAA-3'		
sVEGFR1 Probe	2257	5'-TCCAAATTTAAAAGCACAAGGAATGATTGTACCAC-3'		
U01134				
VEGFR2-FP	791F	5'-CTTCGAAGCATCAGCATAAGAAACT-3'	156 bp	40
VEGFR2-RP	946R	5'-TGGTCATCAGCCCACTGGAT-3'		
VEGFR2 Probe	820	5'-AACCGAGACCTAAAAACCCAGTCTGGGAGT-3'		
AF063658				
NRP1-FP	1831F	5'-CACAGTGGAACAGGTGATGACTTC-3'	112 bp	40
NRP1-RP	1942R	5'-AACCATATGTTGGAAACTCTGATTGT-3'		
NRP1 Probe	1883	5'-CCACAGAAAAGCCCACGGTCATAGACA-3'		
XM_034725				

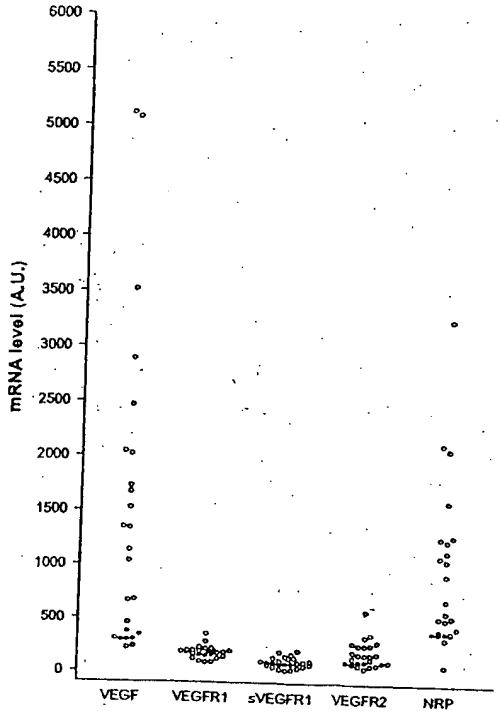


FIGURE 1 – VEGF and VEGF receptors mRNA quantification: scatter of the distribution. Normal brain (black spots) and GBMs (white spots) mRNA levels are expressed as normalized values (as described in Material and Methods: A.U. = arbitrary units). Each point represents the mean of 3 separate experiments.

End point quantitative PCR for MT1-MMP, MMP-2, MMP-9, TIMP-2 mRNA and VEGF mRNA isoforms

MT1-MMP, MMP-2, MMP-9, TIMP-2 and VEGF mRNA isoforms ($VEGF_{189}$, $VEGF_{165}$, $VEGF_{145}$ and $VEGF_{121}$) were measured in 10 ng aliquots of cDNA using Taq polymerase (Takara, Shiga, Japan) and 5 pmol of each primers (Table I). The thermal cycling conditions included 2 min at 95°C for denaturation and then amplification 15 sec at 94°C, 20 sec at 66°C and 20 sec at 72°C (30 sec for VEGF isoforms) with a final incubation 2 min at 72°C. PCR products were resolved on 2% Nusieve 3:1 agarose gels (BioWhittaker, Rockland, MD) and analyzed using a Fluor-S Multilmager (Bio-Rad, Hercules, CA) after ethidium bromide staining. Specific mRNA levels were expressed as the ratio of specific transcripts/28S transcripts. Experiments were repeated at least 3 times in duplicate.

Real-time quantitative PCR for VEGFR-1, sVEGFR-1, VEGFR-2 and NRP1 mRNA

Real-time quantitative RT-PCR analyses for VEGFR-1, sVEGFR-1, VEGFR-2, NRP1 mRNAs and 18S rRNA were carried out using the ABI PRISM 7700 Sequence Detection System instrument and software (PE Applied Biosystems). The sequences of the PCR primer pairs and fluorogenic probes that were used for each gene are shown in Table I. A standard curve was generated by 5-fold serial dilution of placenta cDNA to cover the range of 50,000–80 ng and was run in duplicate during every experiment. For each experimental sample, the amount of target gene was determined from this standard curve. The relative expression level of the target gene was normalized against 18S rRNA to compensate for variation in the quality of RNA and the amount of input cDNA (as described by the manufacturer PE Applied Biosystems in User Bulletin 2). PCR was carried out with the TaqMan Universal PCR Master Mix (Applied Biosystems) using 5 µl of diluted cDNA (equivalent to 10 ng total RNA), 200 nM of the probe, and 400 nM primers in a 25 µl final reaction mixture. After a 2 min incubation at 50°C to allow for UNG cleavage, AmpliTaq Gold was activated by an incubation for 10 min at 95°C. Each of the 40 PCR cycles consisted of 15 sec of denaturation at 95°C and hybridization of probe and primers for 1 min at 60°C.

To confirm amplification specificity, the PCR products were also examined by subsequent 2% agarose gel electrophoresis. Experiments were repeated at least 3 times in duplicate.

Immunohistochemistry for VEGF and MT1-MMP

Sections (4 µm thick) were cut from formalin-fixed, paraffin embedded tumour tissue. They were hydrated through graded alcohols and incubated in H_2O_2 (0.3% 15 min). Sections were autoclaved for 11 min at 126°C in citrate buffer pH6 for antigen retrieval (Dako, Glostrup, Denmark). For double immunostaining sections were incubated in primary monoclonal Ab anti-MT1-MMP (Ab-4) 1:100 (Oncogene Research Products, San Diego, CA) followed by peroxidase-conjugated EnVision (Dako). Immunoreactivity was visualized with 3,3'diaminobenzidine (DAB+, Dako). Sections were then incubated with polyclonal Ab anti-VEGF 1:150 (Santa Cruz, Santa Cruz, CA) for 1 hr at room temperature, followed by alkaline phosphatase-conjugated EnVision (Dako). Immunoreactivity for VEGF was visualized with Fast Red chromogenic substrate (Dako). Single immunostaining was also carried out on serial sections using each primary antibody alone with the corresponding enzyme-chromogene combination. Negative controls were obtained by omitting the primary antibodies.

Gelatin zymography assay

MMP-2 and MMP-9 activities were quantified by gelatin zymography on 2 normal brains and 18 GBMs. Ten cryosections (10 µM) were homogenized in buffer (0.1 M Tris-HCl pH 8.1, 0.4% Triton X-100) and centrifuged for 20 min at 5,000*g*. The pellets were discarded. 25 µg of total protein from homogenate supernatants were mixed with non reducing sample buffer (62.5 mM Tris-HCl, pH 6.8; 2% SDS; 10% glycerol; 0.1% bromophenol blue) and electrophoresed directly on 10% SDS-polyacrylamide gels (SDS-PAGE) containing 0.1% gelatin (w/v).[32] After electrophoresis, gels were washed for 1 hr at room temperature in a 2% (v/v) Triton X-100 solution to remove SDS, transferred to a buffer (50 mM Tris-HCl, pH 7.6, containing 10 mM $CaCl_2$) and incubated for 18 hr at 37°C. Gels were stained for 30 min with 0.1% (w/v) Coomassie brilliant blue G250 in 45% (v/v) methanol/10% (v/v) acetic acid and destained in 10% (v/v) acetic acid/20% (v/v) methanol. Gels were analyzed with Quantity One software (version 4.2.2, Bio-Rad Laboratories, Hercules, CA) after densitometric scanning of the gels using a Fluor-S Multimager (BioRad).

Western blot

MT1-MMP protein levels were analyzed in 2 normal brains and 15 GBMs. Brain extracts (25 µg) were mixed with 1/2 sample buffer [0.25 M Tris (pH6.8), 10% SDS (w/v), 4% sucrose (v/v), 5% β-mercaptoethanol (v/v) and 0.125% bromophenol blue (w/v)] and boiled for 5 min. They were separated on 10% SDS-PAGE gels and transferred to a PVDF filter (NEN, Boston, MA). After blocking with 5% milk (w/v), 0.1% tween 20 (w/v) in PBS for 2 hr at room temperature, membranes were exposed to the primary antibody (10 µg/ml, clone 113-5B7, Ab-4, Oncogene Research Products, San Diego, CA) at 4°C overnight followed by incubation with a horseradish peroxidase-conjugated rabbit anti-mouse antibody (1.3 µg/ml, Dako, Glostrup, Denmark). Signals were detected with an enhanced chemoluminescence (ECL) kit (NEN, Boston, MA). The relative intensities of the immunoreactive bands were analyzed with Quantity One software (version 4.2.2, Bio-Rad

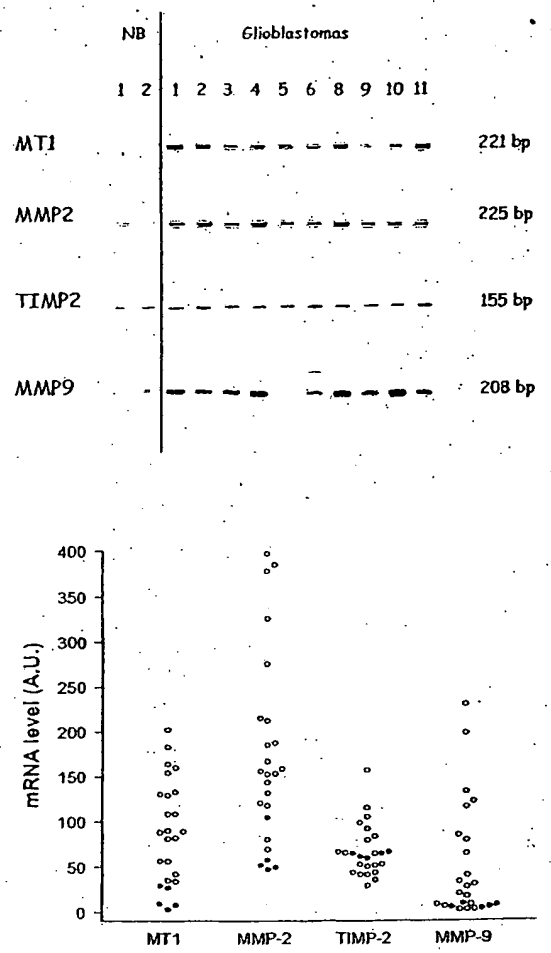


FIGURE 2 – MMPs and TIMP-2 mRNA quantification. (*a*) Representative 2% agarose gels of RT-PCR products for MT1-MMP, MMP-2, TIMP-2 and MMP-9 in 2 normal brains (NB) and 10 GBMs. (*b*) Scatter plots (as described in Fig. 1). Experiment was repeated at least 3 times in duplicate.

Laboratories) after densitometric scanning of the X-ray films using a Fluor-S Multimager (Bio-Rad).

Statistics

VEGF, VEGFRs, MMPs and TIMP-2 expression values in GBMs were correlated using Spearman's test. Correlation was considered significant for 2-tailed p-value < 0.05. Statistical analysis was carried out using the Prism 3.0 software (GraphPad, San Diego, CA).

RESULTS

Expression of VEGF and VEGF receptors

VEGF mRNA was present in normal brains (295–375, arbitrary units; mean = 322) and in all GBM samples (217–5,112; mean = 1,774) as reported previously (Fig. 1).[31] In most GBMs, VEGF mRNA levels were raised 2–15-fold above normal brain values. The most abundant isoform in all cases was $VEGF_{165}$, followed by $VEGF_{121}$, $VEGF_{189}$ and $VEGF_{145}$ (data not shown). VEGFR-1 expression was found at similar levels in GBMs (89–357; mean = 182) and normal controls (154–198; mean = 181). There was no correlation between VEGFR-1 and VEGF mRNA levels (p = 0.35) in GBMs. VEGFR-2 was expressed in all GBMs (48–582; mean = 210) and in 8/20 cases at least twice normal values (87–111; mean = 103). VEGF and VEGFR-2 expressions were correlated significantly (p = 0.0035) in GBMs. NRP1 expression varied broadly between GBMs (75–3,260; mean = 1,061) contrasting with a constant baseline expression in normal controls (383–397; mean = 390). In tumours, NRP1 correlated with VEGFR-2 (p = 0.0119) but not with VEGF (p = 0.084), nor VEGFR-1 (p = 0.066). sVEGFR-1 was expressed at low levels both in normal brains (84–92; mean = 87) and GBMs (25–208; mean = 101). sVEGFR-1, however, was found to correlate with VEGFR-1 (p = 0.0289), VEGFR-2 (p = 0.0029), and NRP1 (p = 0.0027) but not with VEGF (p = 0.053).

Expression of MMPs and TIMP-2

MT1-MMP, MMP-2 and MMP-9 were expressed in both normal brains and GBMs but at much higher levels in the latter (Fig. 2*a*). MT1-MMP mRNA levels were constantly higher in GBMs (34–202; mean = 106) than in normal controls (3–29; mean = 15). MMP-2 and MMP-9 mRNA levels were higher than controls in 18/20 and 14/20 cases respectively (Fig. 2*b*) and correlated with each other (p = 0.0187). MT1-MMP mRNA levels correlated with MMP-2 (p = 0.0008) and MMP-9 (p = 0.005). TIMP-2 had a non-discriminative distribution in relation to the controls. TIMP-2

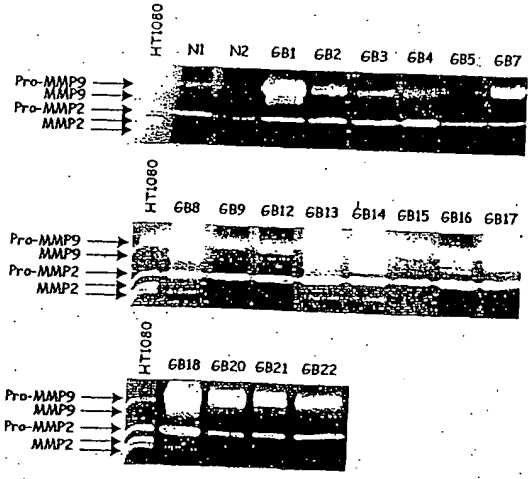


FIGURE 3 – Zymographic analysis of MMP-2 and MMP-9 in tissue extracts either from normal brain (N) or GBMs (GB). Medium conditioned by human HT1080 cells was included as positive control. Positions of pro-MMP-9, MMP-9, pro-MMP-2 and MMP-2 are indicated by arrows.

TABLE II – ZYMOGRAMS AND WESTERN BLOT QUANTIFICATION OF MMPS IN NORMAL BRAIN AND GBMS[1]

	Gelatin zymography				Western blot
	proMMP-9	MMP9	proMMP-2	MMP-2	MT1-MMP
N1	0.41	0.00	1.52	0.00	0.44
N2	0.28	0.00	0.83	0.00	0.47
GB1	9.00	1.25	2.45	0.35	1.58
GB2	4.28	0.00	2.88	0.09	0.47
GB3	1.67	0.00	2.86	0.00	0
GB4	1.40	0.00	4.33	0.03	0
GB5	0.47	0.00	1.15	0.00	ND
GB7	5.29	0.00	1.12	0.00	0
GB8	5.75	3.78	4.63	0.65	0.55
GB9	5.02	0.00	4.09	0.00	0.45
GB12	5.08	0.00	4.79	0.20	3.18
GB13	6.86	4.58	4.93	0.48	1.55
GB14	5.59	6.75	5.53	0.76	0.94
GB15	8.00	0.00	5.13	0.00	ND
GB16	5.55	1.10	5.66	0.00	2.50
GB17	6.61	2.04	6.00	0.00	0
GB18	14.79	4.34	8.20	0.40	1.97
GB20	18.85	0.00	2.12	0.00	0
GB21	16.52	0.00	2.37	0.00	ND
GB22	20.66	0.00	5.11	0.00	0.86

[1]Extracts expressed as arbitrary units. N, normal brain; GB, glioblastoma; ND, not determined.

was correlated with MT1-MMP ($p = 0.0019$) and MMP-2 ($p = 0.0002$) but not with MMP-9 ($p = 0.1408$).

Correlation between MT1-MMP protein and activated MMP-2 and -MMP-9

By gelatin zymography, pro-MMP-2 and pro-MMP-9 were detected in the 18 GBMs and 2 controls examined (Fig. 3, Table II). In most GBMs, levels of these inactive forms were higher than in normal brains and were correlated with their respective mRNA levels (MMP2: $p < 0.0001$; MMP-9 $p = 0.01$) Activated forms of MMP-2 and MMP-9 were not found in normal brain. By contrast, they were present in 8/18 (MMP-2) and 7/18 (MMP-9) GBMs. MT1-MMP protein levels were quantified by Western blot in 15 GBMs and 2 normal brains (Fig. 4, Table II). They were significantly correlated with zymogram-derived activated MMP-2 levels ($p = 0.0226$) but not with activated MMP-9 levels ($p = 0.06$). Interestingly, MT1-MMP protein and mRNA levels were correlated significantly ($p = 0.089$), arguing for a predominantly transcriptional regulation in GBMs.

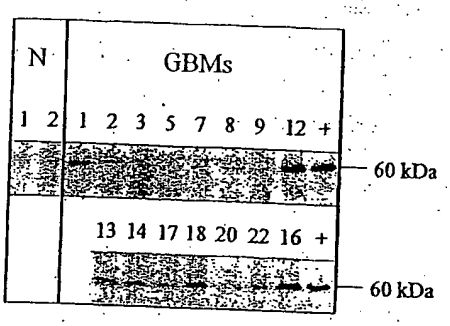


FIGURE 4 – Western blot analysis using the ab4 antibody (clone 113-5B7) raised against the catalytic domain of MT1-MMP. Protein extracts from MT1-MMP transfected A2058 cells (clone SL5,[28]) were used as a positive control (+). MT1-MMP protein is detected in normal brain (N) and GBMs.

TABLE III – CORRELATION BETWEEN VEGF, VEGF RECEPTORS, MMPS AND TIMP-2 EXPRESSED AS p VALUES DERIVED FROM SPEARMAN'S TEST

	MT1-MMP	MMP-2	MMP-9	TIMP-2
VEGF	0.0250	0.0245	0.0053	0.0094
VEGFR-1	0.0073	0.0710	<0.0001	0.4542
VEGFR-2	<0.0001	0.0168	0.0004	0.0153
NRP1	0.0053	0.1334	0.2457	0.5480
sVEGFR-1	0.0313	0.0469	0.0194	0.2563

Correlation between VEGF network and MMPs

mRNA levels of VEGF and VEGF receptors were compared to MT1-MMP, MMP-2, MMP-9 and TIMP-2 (Table III). There was a significant correlation between VEGF expression and MT1-MMP, MMP-2 and MMP-9. A similar correlation was also observed between VEGFR-2 and MMPs. Interestingly, TIMP-2 expression was correlated with VEGF and VEGFR-2 but not with other VEGF receptors.

Immunohistochemistry for VEGF and MT1-MMP

VEGF immunoreactivity was shown in both tumour and endothelial cells, as previously reported (Fig. 5*a,b*).[31] By single immunostaining, MT1-MMP was detected in glioblastoma cells as a diffuse cytoplasmic staining (Fig. 5*c,d*). MT1-MMP positivity was also seen in endothelial cells and perivascular cells (Fig 5*c*). By double immunostaining, we observed the co-localization of VEGF and MT1-MMP in the cytoplasm of numerous tumour cells (Fig. 5*e,f*).

DISCUSSION

GBMs are highly malignant tumours with poor prognosis. They show major microvascular proliferation and express high levels of VEGF.[4–9] VEGF is a strong mitogen for endothelial cells thereby promoting angiogenesis. Previous reports[33,34] have suggested that VEGF also stimulates tumour cell invasion, migration and survival in malignant epithelial cells through an autocrine loop by which overexpression of MMPs induces VEGF secretion and leads to subsequent amplification of cell proliferation and protection against apoptosis. We and others reported previously that in human melanoma and breast carcinoma cells, MT1-MMP upregulates VEGF expression whereas TIMP-2 reduces it.[28,29,35] Therefore the

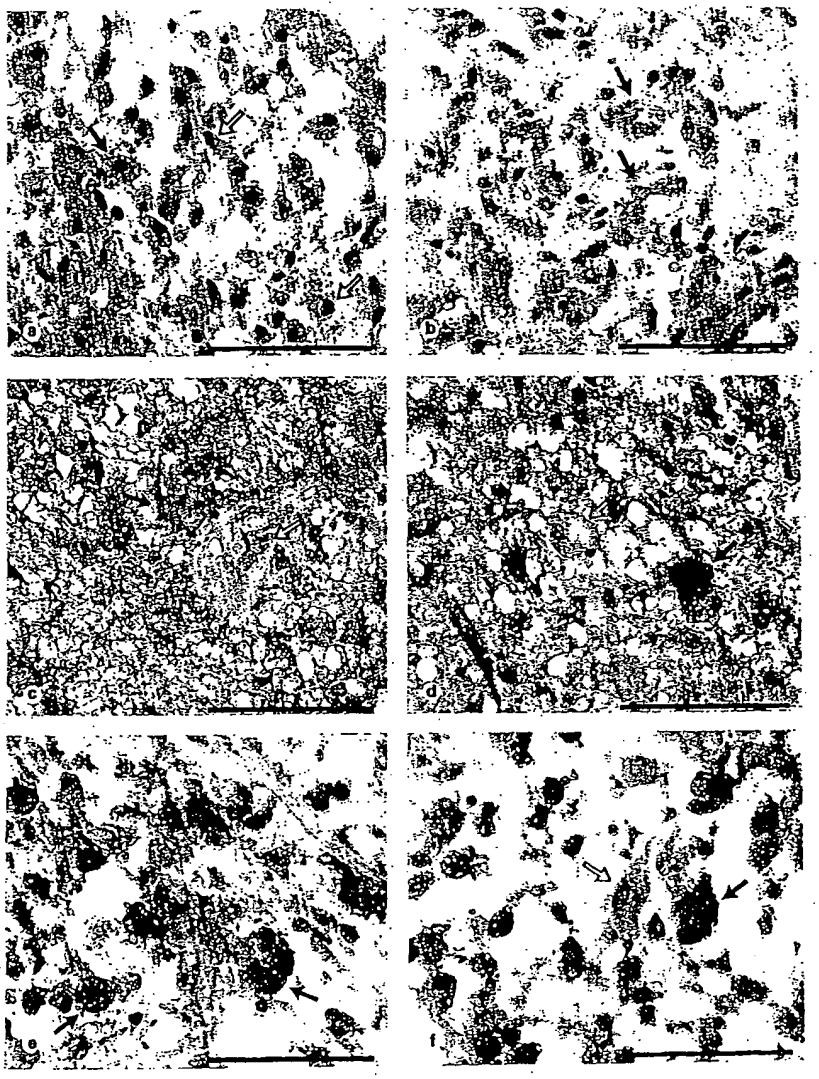


FIGURE 5 – Immunohistochemistry (scale bar = 50 μm). (*a,b*) VEGF positive tumour and endothelial cells (plain arrows) show granular red staining of the cytoplasm. Negative cells (empty arrow) are seen in their close vicinity. (*c*) MT1-MMP positive cells show a strong brown cytoplasmic staining. They include tumours cells (plain arrow) and endothelial cells (empty arrow). (*d*) MT1-MMP positive tumour cells (plain arrow) are mixed with negative cells (empty arrow). (*e,f*) Double staining with VEGF (red) and MT1-MMP1 (brown). Double positive tumour cells (plain arrows) contrast with negative or single weakly positive cell (empty arrow).

pericellular proteolysis mediated by MT1-MMP in GBMs could also induce an autocrine loop resulting in enhanced VEGF expression. In turn, VEGF could act as a paracrine factor on endothelial cells to stimulate angiogenesis or possibly as an autocrine factor promoting glioblastoma cells survival-migration and invasion as demonstrated recently in the various tumour cell culture models.

We compared the expression of VEGF and its receptors with MT1-MMP, MMP-2 and MMP-9 in 20 GBMS and 5 normal brains. The expression of these MMPs was markedly increased in most GBMs with excellent correlation between mRNA and protein levels. MT1-MMP expression has been shown previously to correlate with glioma aggressiveness and its transfection in different tumour cell lines triggers an angiogenic phenotype and promotes tumour growth.[20,28–30,36–38] A majority of GBMs (17/20) also expressed high levels of VEGF, as previously reported, with a strong correlation between VEGF and MT1-MMP gene expression levels. Double immunostaining studies showed co-expression of VEGF and MT1-MMP by the same tumour cells. These data suggest that the transcriptional control of VEGF by MT1-MMP could be operative not only *in vitro* but also *in vivo* in human GBMs.

MT1-MMP could also promote the growth of GBMs by its ability to activate MMP-2 in the presence of low concentration of TIMP-2.[40] Pro-MMP-2 activation occurs after the formation of a ternary complex that contains pro-MMP-2 linked to cell surface MT1-MMP via a TIMP-2 bridge. In accordance with this hypothesis, we found that MMP-2 activation occurred in 8/18 of our GBMs[20,41] among which 7/7 tested for MT1-MMP showed high contents of this protease.

Activated MMP-9 was also found in 7/18 of our GBMs. This is an interesting finding as active MMP-9 is able to mobilize VEGF from its ECM reservoir.[39] Therefore, MMPs could promote VEGF-mediated angiogenesis in GBMs by both transcriptional (MT1-MMP) and post translational (MMP-9) mechanisms.

VEGF binding to VEGFR-2 triggers the proliferation and migration of endothelial cells whereas its binding to VEGFR-1 has

opposite effects on glioblastoma cell lines.[12,42] In our study, VEGF mRNA levels were correlated with VEGFR-2 but not VEGFR-1, NRP1 and sVEGFR-1. Collectively our data suggest that GBMs display a specific and complex pattern of VEGF receptors, transducing VEGF signaling toward cell proliferation and migration.

In conclusion, our study adds to the evidence for an interplay between metalloproteinases and VEGF in human GBMs as previously documented in experimental tumours. Because of its dual ability to activate MMP-2 and to up-regulate VEGF, MT1-MMP might be of central importance in the growth of human glioblastomas and represent an interesting target for anti-cancer treatments.

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to the neurosurgeons of the CHU-Liège (Prof. A. Stevenaert), and CHR-Liège (Prof. J. Born) and Dr. A. Michotte (VUB-Brussels) for their contribution to the collection of cases, to Dr. L. de Leval for her assistance in immunohistochemistry, and to M.R. Pignon for her excellent technical assistance. This work was supported by grants from the Communauté Française de Belgique (Actions de Recherches Concertées), the Commission of European Communities, the Fonds de la Recherche Scientifique Médicale, the Fonds National de la Recherche Scientifique (FNRS, Belgium), the Fédération Belge Contre le Cancer, the Fonds spéciaux de la Recherche (University of Liège), the Centre Anticancéreux près l'Université de Liège, the FB Assurances, the Fondation Léon Frédéricq (University of Liège), the D.G.T.R.E. from the "Région Wallonne", the Fonds d'Investissements de la Recherche Scientifique (CHU, Liège, Belgium), the Interuniversity Attraction Poles (I.U.A.P.) from the Federal Office for Scientific, Technical and Cultural Affairs (O.S.T.C., Brussels, Belgium).

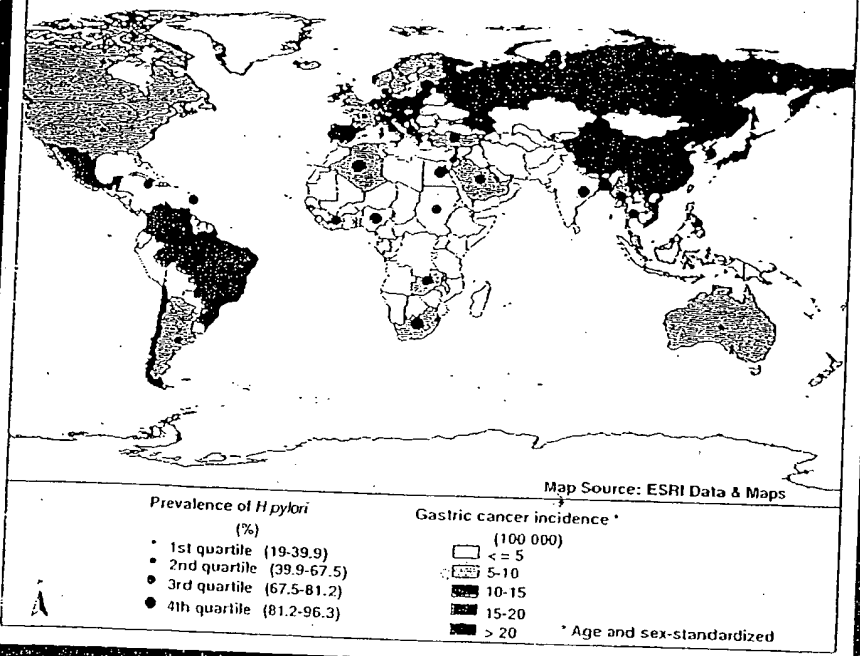
REFERENCES

1. Folkman J, Klagsbrun M. Angiogenic factors. Science 1987;235:442–7.
2. Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. Nature 2000;407:249–57.
3. Plate KH, Breier G, Weich HA, Risau W. Vascular endothelial growth factor is a potential tumour angiogenesis factor in human gliomas in vivo. Nature 1992;359:845–8.
4. Oehring RD, Miletic M, Valter MM, Pietsch T, Neumann J, Fimmers R, Schlegel U. Vascular endothelial growth factor (VEGF) in astrocytic gliomas—a prognostic factor? J Neurooncol 1999;45:117–25.
5. Pietsch T, Valter MM, Wolf HK, von Deimling A, Huang HJ, Cavenee WK, Wiestler OD. Expression and distribution of vascular endothelial growth factor protein in human brain tumors. Acta Neuropathol (Berl) 1997;93:109–17.
6. Nishikawa R, Cheng SY, Nagashima R, Huang HJ, Cavenee WK, Matsutani M. Expression of vascular endothelial growth factor in human brain tumors. Acta Neuropathol (Berl) 1998;96:453–62.
7. Kleihues P, Cavenee WK. Pathology and genetics of tumours of the nervous system. Lyon: IARC Press, 2000. 29–39.
8. Plate KH, Breier G, Weich HA, Mennel HD, Risau W. Vascular endothelial growth factor and glioma angiogenesis: coordinate induction of VEGF receptors, distribution of VEGF protein and possible in vivo regulatory mechanisms. Int J Cancer 1994;59:520–9.
9. Machein MR, Plate KH. VEGF in brain tumors. J Neurooncol 2000;50:109–20.
10. Ding H, Wu X, Roncari L, Lau N, Shannon P, Nagy A, Guha A. Expression and regulation of neuropilin-1 in human astrocytomas. Int J Cancer 2000;88:584–92.
11. Kunkel P, Ulbricht U, Bohlen P, Brockmann MA, Fillbrandt R, Stavrou D, Westphal M, Lamszus K. Inhibition of glioma angiogenesis and growth in vivo by systemic treatment with a monoclonal antibody against vascular endothelial growth factor receptor-2. Cancer Res 2001;61:6624–8.
12. Herold-Mende C, Steiner HH, Andl T, Riede D, Buttler A, Reisser C, Fusenig NE, Mueller MM. Expression and functional significance of vascular endothelial growth factor receptors in human tumor cells. Lab Invest 1999;79:1573–82.
13. Hiratsuka S, Minowa O, Kuno J, Noda T, Shibuya M. Flt-1 lacking the tyrosine kinase domain is sufficient for normal development and angiogenesis in mice. Proc Natl Acad Sci USA 1998;95:9349–54.
14. Soker S, Takashima S, Miao HQ, Neufeld G, Klagsbrun M. Neuropilin-1 is expressed by endothelial and tumor cells as an isoform-specific receptor for vascular endothelial growth factor. Cell 1998;92:735–45.
15. Egeblad M, Werb Z. New functions for the matrix metalloproteinases in cancer progression. Nat Rev Cancer 2002;2:161–74.
16. Pepper MS. Role of the matrix metalloproteinase and plasminogen activator-plasmin systems in angiogenesis. Arterioscler Thromb Vasc Biol 2001;21:1104–17.
17. Sternlicht MD, Werb Z. How matrix metalloproteinases regulate cell behavior. Annu Rev Cell Dev Biol 2001;17:463–516.
18. Sounni NE, Janssen M, Foidart JM, Noel A. Membrane Type-1 Matrix Metalloproteinase and TIMP2 in tumor angiogenesis. Matrix Biol 2003;22:55–61.
19. Belien AT, Paganetti PA, Schwab ME. Membrane-type 1 matrix metalloprotease (MT1-MMP) enables invasive migration of glioma cells in central nervous system white matter. J Cell Biol 1999;144:373–84.
20. Lampert K, Machein U, Machein MR, Conca W, Peter HH, Volk B. Expression of matrix metalloproteinases and their tissue inhibitors in human brain tumors. Am J Pathol 1998;153:429–37.
21. Sato H, Takino T, Okada Y, Cao J, Shinagawa A, Yamamoto E, Seiki M. A matrix metalloproteinase expressed on the surface of invasive tumour cells. Nature 1994;370:61–5.
22. Knauper V, Will H, Lopez-Otin C, Smith B, Atkinson SJ, Stanton H, Hembry RM, Murphy G. Cellular mechanisms for human procollagenase-3 (MMP-13) activation. Evidence that MT1-MMP (MMP-14) and gelatinase a (MMP-2) are able to generate active enzyme. J Biol Chem 1996;271:17124–31.
23. Koshikawa N, Giannelli G, Cirulli V, Miyazaki K, Quaranta V. Role of cell surface metalloprotease MT1-MMP in epithelial cell migration over laminin-5. J Cell Biol 2000;148:615–24.
24. Gilles C, Polette M, Coraux C, Tournier JM, Meneguzzi G, Munaut C, Volders L, Rousselle P, Birembaut P, Foidart JM. Contribution of MT1-MMP and of human laminin-5 gamma2 chain degradation to mammary epithelial cell migration. J Cell Sci 2001;114:2967–76.
25. Kajita M, Itoh Y, Chiba T, Mori H, Okada A, Kinoh H, Seiki M. Membrane-type 1 matrix metalloproteinase cleaves CD44 and promotes cell migration. J Cell Biol 2001;153:893–904.
26. Deryugina EI, Ratnikov B, Monosov E, Postnova TI, DiScipio R, Smith JW, Strongin AY. MT1-MMP initiates activation of pro-MMP-2 and integrin αvβ3 promotes maturation of MMP-2 in breast carcinoma cells. Exp Cell Res 2001;263:209–23.
27. Zhou Z, Apte SS, Soininen R, Cao R, Baaklini GY, Rauser RW, Wang J, Cao Y, Tryggvason K. Impaired endochondral ossification and angiogenesis in mice deficient in membrane-type matrix metalloproteinase I. Proc Natl Acad Sci USA 2000;97:4052–7.
28. Sounni NE, Baramova EN, Munaut C, Maquoi E, Frankenne F, Foidart JM, Noel A. Expression of membrane type 1 matrix metalloproteinase (MT1-MMP) in A2058 melanoma cells is associated with MMP-2 activation and increased tumor growth and vascularization. Int J Cancer 2002;98:23–8.
29. Sounni NE, Devy L, Hajitou A, Frankenne F, Munaut C, Gilles C, Deroanne C, Thompson EW, Foidart JM, Noel A. MT1-MMP expression promotes tumor growth and angiogenesis through an upregulation of vascular endothelial growth factor expression. FASEB J 2002;16:555–64.
30. Deryugina EI, Soroceanu L, Strongin AY. Upregulation of vascular endothelial growth factor by membrane-type 1 matrix metalloproteinase stimulates human glioma xenograft growth and angiogenesis. Cancer Res 2002;62:580–8.
31. Munaut C, Boniver J, Foidart JM, Deprez M. Macrophage migration inhibitory factor (MIF) expression in human glioblastomas correlates with VEGF expression. Neuropathol Appl Neurobiol 2002;28:452–60.
32. Munaut C, Noel A, Weidle UH, Krell HW, Foidart JM. Modulation of the expression of interstitial and type-IV collagenases in coculture of HT1080 fibrosarcoma cells and fibroblasts. Invasion Metastasis 1995;15:169–78.
33. Bachelder RE, Crago A, Chung J, Wendt MA, Shaw LM, Robinson G, Mercurio AM. Vascular endothelial growth factor is an autocrine survival factor for neuropilin-expressing breast carcinoma cells. Cancer Res 2001;61:5736–40.
34. Chung J, Bachelder RE, Lipscomb EA, Shaw LM, Mercurio AM. Integrin (α6β4) regulation of eIF-4E activity and VEGF translation: a survival mechanism for carcinoma cells. J Cell Biol 2002;158:165–74.
35. Hajitou A, Sounni NE, Devy L, Grignet-Debrus C, Lewalle JM, Li H, Deroanne CF, Lu H, Colige A, Nusgens BV, Frankenne F, Maron A,

et al. Downregulation of vascular endothelial growth factor by tissue inhibitor of metalloproteinase-2: effect on in vivo mammary tumor growth and angiogenesis. Cancer Res 2001;61:3450–7.
36. Forsyth PA, Laing TD, Gibson AW, Rewcastle NB, Brasher P, Sutherland G, Johnston RN, Edwards DR. High levels of gelatinase-B and active gelatinase-A in metastatic glioblastoma. J Neurooncol 1998;36:21–9.
37. Nakada M, Nakamura H, Ikeda E, Fujimoto N, Yamashita J, Sato H, Seiki M, Okada Y. Expression and tissue localization of membrane-type 1, 2, and 3 matrix metalloproteinases in human astrocytic tumors: Am J Pathol 1999;154:417–28.
38. Kachra Z, Beaulieu E, Delbecchi L, Mousseau N, Berthelet F, Moumdjian R, Del Maestro R, Beliveau R. Expression of matrix metalloproteinases and their inhibitors in human brain tumors. Clin Exp Metastasis 1999;17:555–66.
39. Bergers G, Brekken R, McMahon G, Vu TH, Itoh T, Tamaki K, Tanzawa K, Thorpe P, Itohara S, Werb Z, Hanahan D. Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis. Nat Cell Biol 2000;2:737–44.
40. Strongin AY, Collier I, Bannikov G, Marmer BL, Grant GA, Goldberg GI. Mechanism of cell surface activation of 72-kDa type IV collagenase. Isolation of the activated form of the membrane metalloprotease. J Biol Chem 1995;270:5331–8.
41. Nakada M, Kita D, Futami K, Yamashita J, Fujimoto N, Sato H, Okada Y. Roles of membrane type 1 matrix metalloproteinase and tissue inhibitor of metalloproteinases 2 in invasion and dissemination of human malignant glioma. J Neurosurg 2001;94:464–73.
42. Millauer B, Wizigmann-Voos S, Schnurch H, Martinez R, Moller NP, Risau W, Ullrich A. High affinity VEGF binding and developmental expression suggest Flk-1 as a major regulator of vasculogenesis and angiogenesis. Cell 1993;72:835–46.

INTERNATIONAL
Journal of Cancer

Helicobacter pylori and gastric cancer: facing the enigmas



SUBSCRIPTION INFORMATION

International Journal of Cancer (Print ISSN 0020-7136; Online ISSN 1097-0215) is published 30 times a year, semi-monthly with extra issues in January, March, May, July, September, and November, by Wiley-Liss, Inc., through Wiley Subscription Services, Inc., a Wiley Company. **Send subscription inquiries** in care of John Wiley & Sons, Inc., Attn: Journals Admin Dept UK, 111 River St., Hoboken, NJ 07030, (201) 748-6645.
Advertising inquiries should be addressed to Advertising Department, c/o John Wiley & Sons, Inc., 111 River St., Hoboken, NJ 07030. Telephone: (201) 748-6921.
Offprint sales and inquiries should be directed to the Customer Service Department, in care of John Wiley & Sons, 111 River St., Hoboken, NJ 07030. Telephone: (201) 748-8776.
Subscription price: Volumes 103–107, 2003 (30 issues). Print only: $2,495 worldwide. Electronic only: $2,495 worldwide. A combination price of $2,620 worldwide includes the subscription in both electronic and print formats. All subscriptions containing a print element, shipped outside the U.S., will be sent by air. Payment must be made in U.S. dollars drawn on U.S. bank. Periodicals postage paid at Hoboken, NJ and at additional mailing offices. Postmaster: send address changes to INTERNATIONAL JOURNAL OF CANCER, Subscription Distribution, c/o John Wiley & Sons, Inc., 111 River St., Hoboken, NJ 07030.
Change of address: Please forward to the subscriptions address listed above 6 weeks prior to move; enclose present mailing label with change of address. **Claims for missing issues**: Claims for undelivered copies will be accepted only after the following issue has been received. Please enclose a mailing label or cite your subscriber reference number. Missing copies will be supplied when losses have been sustained in transit and where reserve stock permits. Send claims in care of John Wiley & Sons, Inc., Attn: Journals Admin Dept UK, 111 River St., Hoboken, NJ 07030.
Indexed by: EMBASE/Excerpta Medica • Current Contents/Life Sciences • Science Citation Index • Scisearch • BIOSIS Data Base • Index Medicus • Cambridge Scientific Abstracts • Chemical Abstracts • Reference Update • Smoking and Health Database.
This journal is printed on acid-free paper.

INTERNATIONAL JOURNAL OF CANCER

The *International Journal of Cancer* is published for the International Union Against Cancer by Wiley-Liss, Inc., a division of John Wiley & Sons, Inc. Five volumes are issued annually, each consisting of six numbers.

Abstracting and other journals may reprint the summaries of articles without requesting authorization. Authors alone are responsible for views expressed in signed articles. The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the International Union Against Cancer.

References: When quoting from the *International Journal of Cancer*, please use the official abbreviation: *Int. J. Cancer*

89: Leuk Lymphoma. 2003 Aug;44(8):1385-94. Related Articles, Links

Real-time quantitative RT-PCR of cyclin D1 mRNA in mantle cell lymphoma: comparison with FISH and immunohistochemistry.

Hui P, Howe JG, Crouch J, Nimmakayalu M, Qumsiyeh MB, Tallini G, Flynn SD, Smith BR.

Department of Laboratory Medicine, Yale University School of Medicine, 333 Cedar Street, P.O. Box 208035, New Haven, CT 06520-8035, USA.

Presence of the balanced translocation t(11;14)(q13;q32) and the consequent overexpression of cyclin D1 found in mantle cell lymphoma (MCL) has been shown to be of important diagnostic value. Although many molecular and immunohistochemical approaches have been applied to analyze cyclin D1 status, correlative studies to compare different methods for the diagnosis of MCL are lacking. In this study, we examined 39 archived paraffin specimens from patients diagnosed with a variety of lymphoproliferative diseases including nine cases meeting morphologic and immunophenotypic criteria for MCL by: (1) real-time quantitative RT-PCR to evaluate cyclin D1 mRNA expression; (2) dual fluorescence in situ hybridization (FISH) to evaluate the t(11;14) translocation in interphase nuclei; and (3) tissue array immunohistochemistry to evaluate the cyclin D1 protein level. Among the nine cases of possible MCL, seven cases showed overexpression of cyclin D1 mRNA (cyclin D1 positive MCL) and two cases showed no cyclin D1 mRNA increase (cyclin D1 negative "MCL-like"). In six of seven cyclin D1 positive cases, the t(11;14) translocation was demonstrated by FISH analysis; in one case FISH was unsuccessful. Six of the seven cyclin D1 mRNA overexpressing cases showed increased cyclin D1 protein on tissue array immunohistochemistry; one was technically suboptimal. Among the two cyclin D1 negative MCL-like cases, FISH confirmed the absence of the t(11;14) translocation in both cases. All other lymphoproliferative diseases studied were found to have low or no cyclin D1 mRNA expression and were easily distinguishable from the cyclin D1 overexpressing MCLs by all three techniques. In addition, to confirming the need to assess cyclin D1 status, as well as, morphology and immunophenotyping to establish the diagnosis of MCL, this study demonstrates good correlation and comparability between measure of cyclin D1 mRNA, the 11;14 translocation and cyclin D1 protein.

Publication Types:

- Evaluation Studies

PMID: 12952233 [PubMed - indexed for MEDLINE]